J. Physiol. (1966), **182**, *pp.* 268–296
With 14 *text-figures*
Printed in Great Britain

THE EXCITATORY SYNAPTIC ACTION OF CLIMBING FIBRES ON THE PURKINJE CELLS OF THE CEREBELLUM

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(*Received* 13 *August* 1965)

SUMMARY

1. A single climbing fibre makes an extraordinarily extensive synaptic contact with the dendrites of a Purkinje cell. Investigation of this synaptic mechanism in the cerebellum of the cat has been based on the discovery by Szentagothai & Rajkovits (1959) that the climbing fibres have their cells of origin in the contralateral inferior olive.

2. Stimulation in the accessory olive selectively excites fibres that have a powerful synaptic excitatory action on Purkinje cells in the contralateral vermis, evoking a repetitive spike discharge of 5–7 msec duration. Almost invariably this response had an all-or-nothing character. In every respect it corresponds with the synaptic action that is to be expected from climbing fibres.

3. Intracellular recording from Purkinje cells reveals that this climbing fibre stimulation evokes a large unitary depolarization with an initial spike and later partial spike responses superimposed on a sustained depolarization.

4. Typical climbing fibre responses can be excited, but in a much less selective manner, by stimulation of the olive-cerebellar pathway in the region of the fastigial nucleus, there being often a preceding antidromic spike potential of the Purkinje cell under observation.

5. Impaled Purkinje cells rapidly deteriorate with loss of all spike discharge, the climbing fibre response being then reduced to an excitatory post-synaptic potential. This potential shows that stimulation of the inferior olive may evoke two or more discharges at about 2 msec intervals in the same climbing fibre. The complexity of neuronal connexions in the inferior olive is also indicated by the considerable latency range in responses.

6. A further complication is that, with stimulation in the region of the fastigial nucleus, the initial direct climbing fibre response is often followed by a reflex discharge, presumably from the inferior olive, which resembles

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the responses produced by inferior olive stimulation in being often repetitive.

7. Typical climbing fibre responses have been evoked by peripheral nerve stimulation and frequently occur spontaneously.

8. An account is given of the way in which the responses evoked by climbing fibres in the individual Purkinje cells can account for the potential fields that an inferior olive stimulus evokes on the surface and through the depth of the cerebellar cortex.

9. By the application of currents through the recording intracellular electrode it has been possible to effect large changes in the excitatory post-synaptic potential produced by a climbing fibre, it being diminished and even reversed with depolarizing currents and greatly increased by hyperpolarizing currents.

INTRODUCTION

The climbing fibres of the cerebellar cortex were discovered by Ramón y Cajal (1888, 1890) and further investigated and described by him (Ramón y Cajal & Illera, 1907; Ramón y Cajal, 1911) and many other neurohistologists of the classical era. More recently Scheibel & Scheibel (1954) have made a thorough Golgi study of climbing fibres and have reported more extensive synaptic relations than those previously described. To some extent these findings have been confirmed by Fox (1962) and by Szentágothai (1964, 1965); and they will be critically examined in the discussion in relation to the findings described in this paper. In the classical description of Ramón y Cajal (cf. Fig. 1) each climbing fibre traverses the granular layer of the cerebellar cortex without branching. After passing by the body of a Purkinje cell it breaks into branches that twine around each of the large primary and secondary dendrites of the Purkinje cell, but avoid the small spiny branchlets. This very extensive and intimate contact was assumed to form a powerful synaptic mechanism, and recently the structural details of this synapse have been demonstrated by electron microscopy (Szentágothai, 1964, 1965; Palay, personal communication; C. A. Fox, personal communication).

The study of the origin of the climbing fibres by the conventional degenerative methods has proved to be extremely difficult technically. As a consequence several different origins have been proposed for the climbing fibres in a series of publications that have been critically reviewed by Szentágothai & Rajkovits (1959). On the general grounds of the widespread distribution of the olivo-cerebellar fibres Dow (1942) suggested that the climbing fibres originated in the inferior olive, all other cerebellar inputs being via mossy fibres. But it was not until the very careful degenerative study by Szentágothai & Rajkovits (1959), that a

sound experimental basis was provided for the olivary origin of the climbing fibres, as illustrated in Fig. 1.

The present investigation has utilized this discovery of Szentágothai & Rajkovits, it being assumed that climbing fibres could be selectively excited by an electrode (I.O in Fig. 1) buried in the inferior olivary nucleus—actually in the accessory olives for the fibres projecting to the anterior lobe of the cerebellar vermis (Brodal, 1954). Stimulation of the inferior olive has been found to evoke electrical responses widely dispersed over the cerebellum (Dow, 1939; Combs, 1956; Jansen, 1957). The dominant potential was a surface negative wave with a latency of 3–5 msec, but considerable variations were reported in the potentials led from different parts of the cerebellum. Certainly the later positive potentials are probably due in large part to the spread of stimulus to other structures adjacent to the inferior olive. We have employed both extracellular and intracellular micro-electrode recording from Purkinje cells in establishing that single olivo-cerebellar fibres have a uniquely powerful excitatory action on Purkinje cells, exactly as would be expected for climbing fibres (cf. Fig. 1). In addition to the activation of climbing fibres by stimulation of the inferior olive, these fibres have also been stimulated both directly and reflexly by an electrode in the proximity of the fastigial nucleus. A preliminary account has already been published (Eccles, Llinás & Sasaki, 1964*a*).

METHODS

Cats ranging in weight from 3 to 3·5 kg were anaesthetized with pentobarbital sodium (30 mg/kg) administered intraperitoneally. The cerebellar vermis was exposed by a craniotomy which extended from the bony tentorium to the lambdoidal ridge of the occipital bone. In most instances, the craniotomy was extended to the foramen magnum. This procedure

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Legend to Fig. 1

Fig. 1. Diagram of the experimental arrangement. A small portion of the cerebellar cortex with its three main layers, the molecular (M.L.) granular (G.L.) and Purkinje (P.L.) is schematically drawn, and their corresponding depths are marked in the right side of the figure. The two inputs are shown to the Purkinje cell (P): the climbing fibre (CF) with its extraordinarily extended synaptic contacts on the dendrites of the Purkinje cell; and the mossy fibre (M.F.), granulae cell (G.C.), parallel fibre (P.F.) pathway. The climbing fibres are the mode of termination of the olivo-cerebellar pathway (O.C.P). (see text) which arises in the contralateral inferior olive (I.O.) and reaches the cerebellar cortex through the ipsilateral inferior peduncle (inf. ped.). The Purkinje cell axon (P.A.) has collaterals (P.A.C.) which ramify in the granular and the molecular layer of the cortex forming the infra and supra ganglionic plexus respectively. Stimulating electrodes were located in the inferior olive (I.O.), juxta-fastigial region (J.F.) and the surface of the cortex (*LOC*). The recordings were made from the surface of the cortex with a ball electrode (S.R.) and in depth with micro-electrodes.

exposed two or three folia rostral to the fissura prima and a large proportion of the posterior lobe folia. Recordings were usually restricted to the culmen and lobus simplex. Laterally, the craniotomy extended the whole width of the vermal region. This ample exposure was needed to allow the selection of a recording site in the area of maximum response to the multiple inputs under study. The craniotomy was performed with bone nibblers under continuously running warm Ringer's solution in order to prevent air embolism.

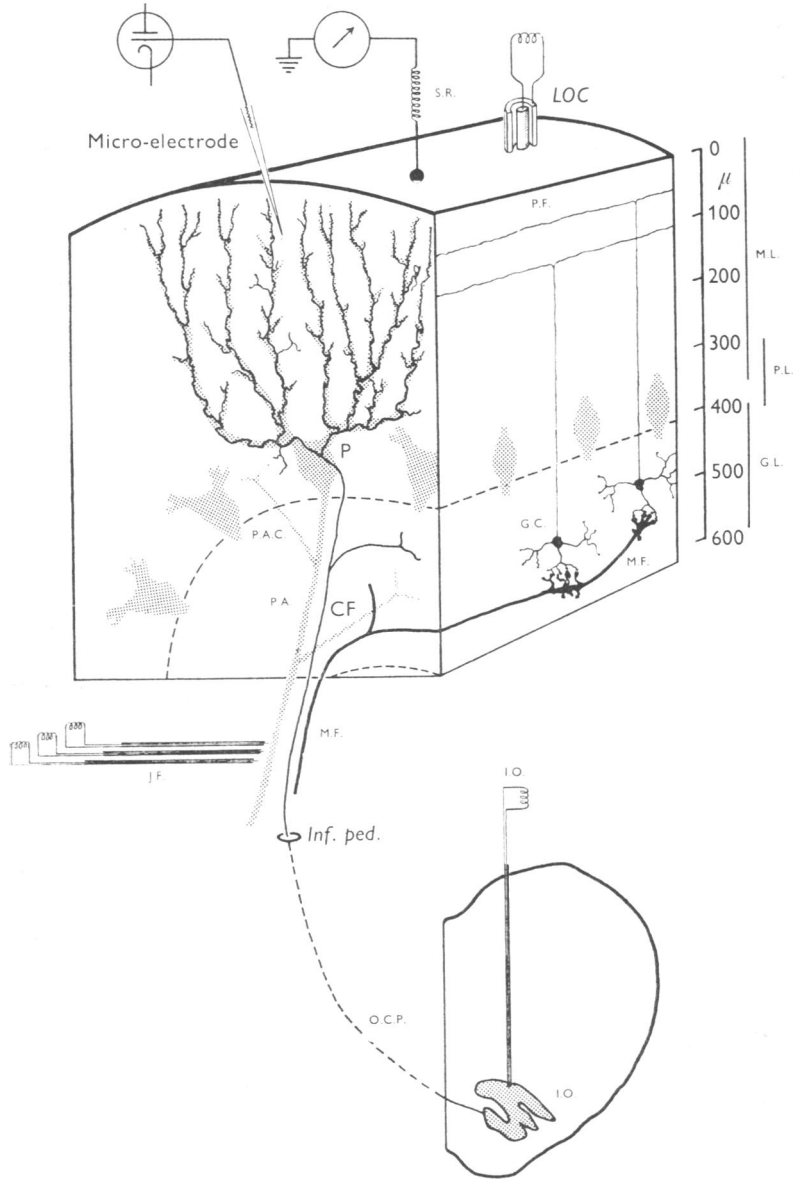

Fig. 1. For legend see opposite page.

Figure 1 illustrates diagrammatically the general experimental arrangements. Monopolar surface recordings were obtained with a spring mounted silver ball electrode (S.R.) that was insulated with P.V.C. except for the very small area in contact with the cortex. The stimulating juxta-fastigial electrode (J.F.) consisted of three independent bipolar concentric electrodes. A No. 30 hypodermic needle tubing was insulated with epoxy-resin up to 500 μ from the tip and an enamel-insulated internal nichrome wire No. 48 (S.W.G.) protruded about 700 μ from the end of the tube with a 300 μ length of uninsulated area. These three electrodes were cemented together so that their tips were 1·5 mm apart, thus allowing a very localized stimulus at each of those three points in the cerebellar white matter. Single bipolar electrodes were placed in the lateral cuneate nucleus and in or close to the accessory olive nuclei. This latter electrode will henceforth be referred to as the inferior olive electrode (I.O.). A very short flat-ended concentric electrode (2 mm in length and outside diameter 0·8 mm) mounted on springy wire, was utilized to stimulate the surface of the cortex (*LOC*). This surface electrode allows a very localized region of stimulation, thus producing a well-defined 'beam' of activation in the parallel fibres. The superficial and deep radial nerves were stimulated with platinum electrodes under an oil pool.

Once the optimum responses from I.O. stimulation were recorded with the surface electrode, the latter was removed to a nearby region of the same folium; and, after covering the exposed area of the cerebellum with an agar gel, a micro-electrode for depths recording was lowered into the region of maximum response. Micro-electrodes were filled with 4 M NaCl for the laminar field analysis (2 MΩ average d.c. resistance) and with 3 M KCl or 2 M K citrate for the intracellular recordings (10 MΩ average d.c. resistance). In order to minimize the movement of the cerebellar cortex, a bilateral pneumothorax was performed after the animal had been paralysed with gallamine triethiodide (Flaxedil) and artificially ventilated. A bridge circuit (Araki & Otani, 1955) with a 100 MΩ resistor facing the grid of the cathode follower was used to pass current through the impaled cell. An RC coupled amplifier with a time constant of 3–10 sec was used for the potential recording. The current measurement was made across a 180 kΩ resistor using a differential d.c. amplifier of high input impedance. The membrane potential was constantly monitored with a d.c. amplifier feeding a voltmeter.

In the initial stages of this work histological sections (haematoxylin eosin) were made to verify the stimulating electrode placements in the inferior olive. Once the 'typical' olivary response was established, however, only occasional checks were deemed necessary, because the cerebellar responses evoked by the olivary stimulation were so characteristic.

RESULTS

Stimulation of inferior olivary nucleus

Potential profile in cerebellar cortex. Figure 2*A* shows a series of potentials evoked by a stimulus applied through an electrode in the inferior olive and recorded by a micro-electrode in the contralateral vermis at the indicated depths below the surface. At all depths there was an initial negative wave with a fairly steep rise and a slower decline on which were superimposed small irregular spike-like potentials. This negative wave declined to a small slow positive wave, which was relatively larger at the deeper levels.

In five of our twelve experiments on potential profiles this positive wave was dominant at the deeper levels (cf. Fig. 2*B*, *C*, *D*), there being only a fraction of an initial negativity below 250 μ. When applying stimuli to

structures like the inferior olive that are surrounded by neural pathways of diverse function, there is always a danger that the observed responses are contaminated by spread of stimulation. The diversity of the potential profiles could arise in this way.

Figure 2*B–D* illustrates an experimental test for this possible distortion. Three strengths of inferior olive stimulation (3·0, 2·1 and 1·5 times threshold) were applied at each position of the micro-electrode. At the superficial levels the respective potentials differed in size, but not significantly in configuration and time course, there being a large initial negative wave and a later positive wave, which became dominant with depths from 230 μ downwards. However, at 330 and 430 μ the strongest stimulus (*B*) set up a positive wave that was relatively much larger, so it seems likely that this deep positive wave in part was produced by stimulation of structures adjacent to the inferior olive, such as the reticulo-cerebellar fibres from the lateral reticular nucleus, as will be described in a later paper. Otherwise the potential profiles of Figs. 2*B–D* may be regarded as being produced by stimulation of olivo-cerebellar fibres.

The latency of the negative wave in the superficial levels of the cerebellar cortex is so brief, 3·9–6·8 msec in six experiments, that it must be produced by a direct olivo-cerebellar pathway, which has been shown by Szentágothai & Rajkovits (1959) to be exclusively a climbing fibre pathway. Hence the potential profiles of Fig. 2 can be regarded as being produced by impulses in climbing fibres and composed of both the action potentials of these impulses and the synaptic potentials generated by them.

The large extracellular negative potentials at levels from 30 to 200 μ in Fig. 2*A–D* exactly correspond to the potentials that would be expected for a depolarizing synaptic action of the climbing fibre impulses on the Purkinje cells, for this range includes the levels of greatest synaptic density. The decline in negative potential and even its reversal to a positive wave below 200 μ in *B–D* are attributable to the somata and axons of the Purkinje cells acting as passive sources for the active sinks on the main dendrites. In Fig. 2 it can be seen that, with progressively more superficial levels, there is correspondingly a longer latency of the initial negative wave, which is of course attributable to the increasing conduction time in the climbing fibres. An approximate conduction velocity of 0·15–0·5 m/sec was, on this basis, computed for the fine terminal branches of these fibres in four experiments.

The diphasic negative-positive potential produced by inferior olive stimulation at very superficial levels (Fig. 2) and actually on the surface (Fig. 3*E*) is in good agreement with the potential (usually a negative then positive wave) that such a stimulation was observed to produce with surface recording (Dow, 1939; Jansen, 1957).

As described in a later paper there are subsidiary weak synaptic excitatory actions of climbing fibres on basket cells and on Golgi cells, findings which are in accord with histological observations (Scheibel & Scheibel, 1954; Szentágothai, 1965); but these actions could make only a negligible contribution to the potential field, and in any case would give a much deeper negativity (about 400 μ) than that actually observed. However, there is the further possibility that the inhibitory synaptic actions of these excited basket (Andersen, Eccles & Voorhoeve, 1964) and Golgi cells (Eccles, Llinás & Sasaki, 1964*b*) may contribute to the positive potential at the deeper levels in Fig. 2.

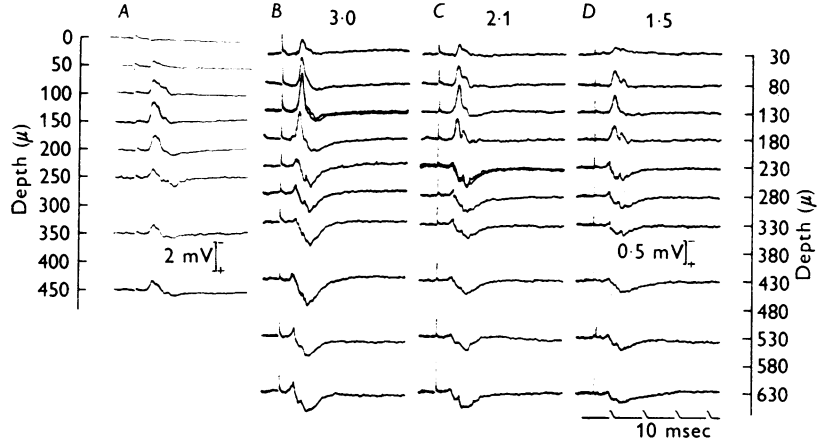

Fig. 2. Laminar analysis of the field potential produced in the contralateral cerebellar cortex by stimulation of the inferior olive. Two experiments are illustrated, *A* (left depth scale) and *B*, *C*, *D* (depth scale of the right). Especially clear in record *A* is the difference in latency of the early negative component of the field at the different depths, which is interpreted to be the conduction time for the activity in the climbing fibres. Note the different voltage calibration for records *A* and *B*, *C*, *D*, but the time scale is the same. Timing of stimuli indicated by initial downward artifact in *A* and by initial upward artifact in *B*, *C* and *D*.

Since the climbing fibres are slender and rather sparsely distributed, it would be expected that the action potentials in these fibres likewise would make a negligible contribution to the observed potential field, particularly as their range of conduction velocities would result in a temporal dispersion over several milliseconds. Furthermore, they would produce a potential field having an initial positivity and later negativity, not the initial negative potential actually observed.

It seems therefore justifiable to conclude that the potential profiles of Figs. 2*A–D* are generated by the synaptic excitatory action of climbing fibre impulses on the dendrites of Purkinje cells, and that they are in part due to excitatory post-synaptic potentials, and in part to the impulses

generated thereby. This provisional conclusion will be corroborated by the more analytical investigations described below, such as selective extracellular and intracellular recording from individual Purkinje cells.

Extracellular recording of spike potentials. In Fig. 3*A* the inferior olive stimulus evoked a complex series of spike potentials at a depth of 250 μ in the cortex of the cerebellar vermis. By careful adjustment of stimulus strength this whole complex was displayed in Fig. 3*B* as a unitary response that was superimposed on a low background potential. There was an initial large diphasic spike potential (positive-negative) and a succession of spike potentials of the same polarity but of various smaller sizes. For example, in Fig. 3*B* there were four superimposed traces, two below threshold and two above. The two latter gave four successive superimposed spikes.

Since the Purkinje cells represent the only efferent system of the cerebellar cortex, a stimulus applied to the juxta-fastigial region (cf. Fig. 1) would be expected to excite the axons of Purkinje cells and of no other cells in the cerebellar cortex; and in Fig. 3*C* there is initially an antidromic spike potential having a configuration closely resembling the initial spike evoked from the inferior olive; hence it can be concluded that this spike was evoked in the same Purkinje cell. Each subsequent spike potential in Fig. 3*A*, *B*, *C* had the same configuration as the antidromic spike in *C*, and the smaller size is attributable to background depolarization of the Purkinje cell and to relative refractoriness at that high frequency of response; hence these spikes also were produced by the same Purkinje cell. The all-or-nothing property of the spike complex in Fig. 3*B* establishes that a single neural element coming from the inferior olive had a powerful excitatory influence on this Purkinje cell, the synaptic depolarization being so prolonged that it generated three or four impulses, and so large that the spike mechanism was greatly depressed. From the histological pictures of the climbing fibre synapse it would be expected to have such a powerful action.

Other examples of unitary responses of Purkinje cells are shown in Fig. 3*D* (*E* is the simultaneous surface recording), where the initial spike was followed by a relatively negative wave with small superimposed spikes, and also in Fig. 3*F*, *G*, where two spikes of the Purkinje cell were superimposed on a relatively large, slow positive wave. The whole assemblage in *F* and *G* represents an all-or-nothing unit superimposed on a field potential that is well shown in *F*. It will be appreciated that in Fig. 3*B*, *D*, *F*, *G* the unitary potential complex is superimposed upon a background potential generated by more distant Purkinje cells. When the recording electrode is superficial, the unitary potentials are characterized by spikes superimposed on a large slow negative potential. On the other hand, when

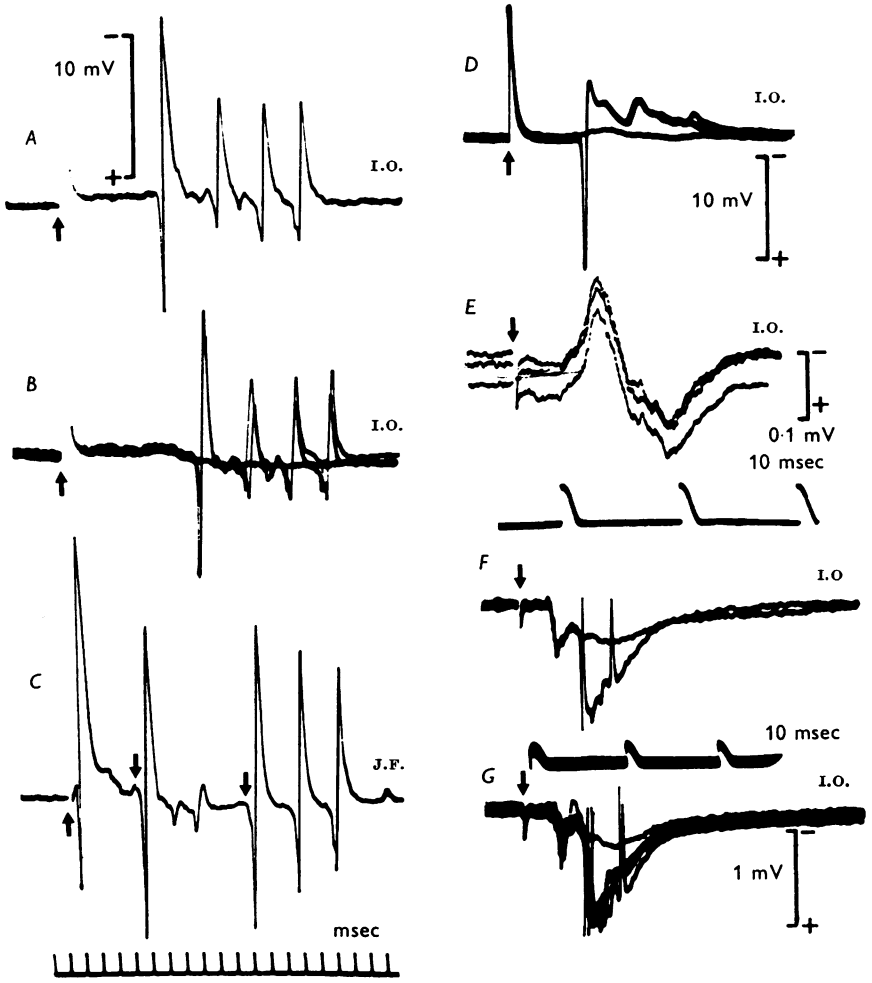

Fig. 3. Inferior olive activating of Purkinje cells. An extracellular 'giant' spike from a Purkinje cell is illustrated in *A*, *B* and *C*. In *A* and *B* the cell is fired by I.O. stimulation. In *B* four superimposed sweeps show the all-or-nothing character of this response, two of the four stimuli being subthreshold. The spikes were so regular that they were superimposed. In *B* the latency was longer than in *A* because the stimulus was weaker (see discussion for Fig. 6). In *C*, following the antidromic invasion of the cell by J.F. stimulation (up-going arrow), there are two sets of responses (down-going arrows); the first was produced by the direct stimulation of the incoming climbing fibre, the second by reflex activation via the inferior olive (see Fig. 10). In *D* three superimposed sweeps show an all-or-nothing response of a Purkinje cell evoked by I.O. stimulation; *E* shows a simultaneous recording from the surface of the cerebellar cortex (S.R. in Fig. 1). *F* and *G* are similar all-or-nothing responses of another Purkinje cell by I.O. stimulation, *G* illustrating the constancy of the response. For all records the first arrow signals stimulus artifact. Time scale of *C* and voltage scale of *A* apply to records *A*, *B* and *C*. Time scale for *D* and *E* is shown in *E*.

it is relatively deep (300 μ in Fig. 3*F*, *G*), the unitary potential is formed by spikes superimposed on a slow positive potential.

Intracellular recording from Purkinje cells. The intracellularly recorded responses to an inferior olive stimulus are in good agreement with the extracellular response. For example, the first spike in Fig. 4*B* indicates antidromic invasion from a juxta-fastigial stimulation and so provides Purkinje cell identification; and, in response to an inferior olive stimulation (Fig. 4*A*), there was an initial spike with a latency of 5·8 msec and a later large slow potential on which were superimposed several small spikes. The whole spike complex evoked by inferior olive stimulation

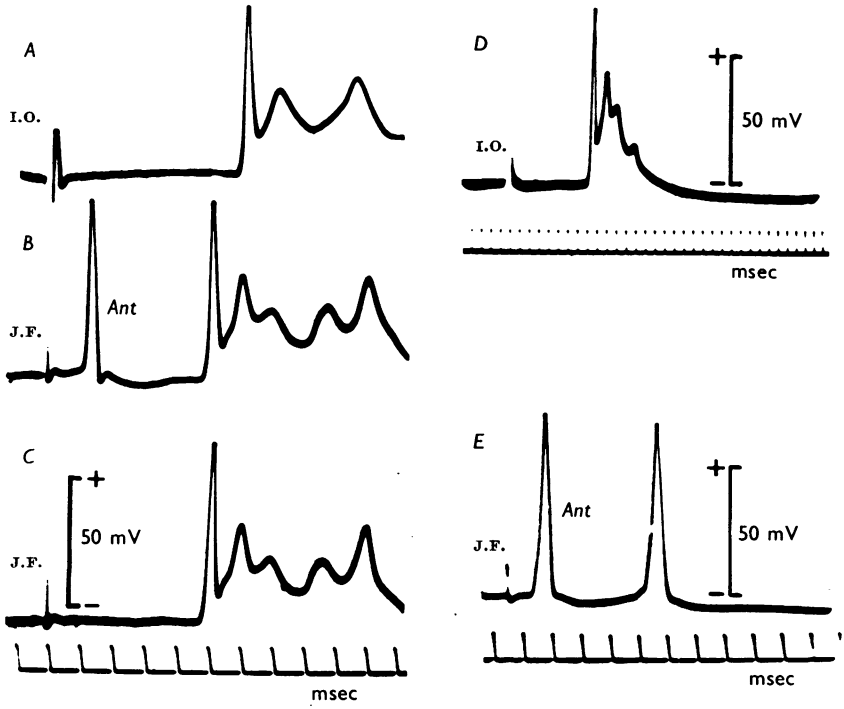

Fig. 4. Intracellular records from Purkinje cells activated from the inferior olive and by juxta-fastigial stimulation. *B* and *C* responses were evoked by J.F. stimulation; in *B* the cell was also antidromically activated, while in *C* the stimulus strength was subthreshold for antidromic invasion. In both there was a later spike followed by a prolonged complex depolarization that was virtually identical. I.O. stimulation in *A* evoked in the same cell a similar complex depolarization, the only difference being its longer latency. *D* and *E* are recordings from another Purkinje cell, *D* being the intracellular response evoked by I.O. stimulation at a slow sweep speed and *E* shows the antidromic activation by J.F. stimulation. The second action potential in *E* is produced by mossy fibre activation through the granule cell-parallel fibre pathway. Time scale and voltage calibration of *C* apply to records *A*, *B* and *C*.

usually had a duration of 5–7 msec. Figure 4*D* gives another example of the initial spike and later EPSP and spike complex evoked in a Purkinje cell which was identified by antidromic invasion in response to a juxta-fastigial stimulus (Fig. 4*E*). The later spike of the Purkinje cell in Fig. 4*E* will be shown in a subsequent paper to be generated by excitation of mossy fibres that in turn excite granule cells and so the parallel fibres with their multitude of excitatory synapses on the Purkinje cell dendrites.

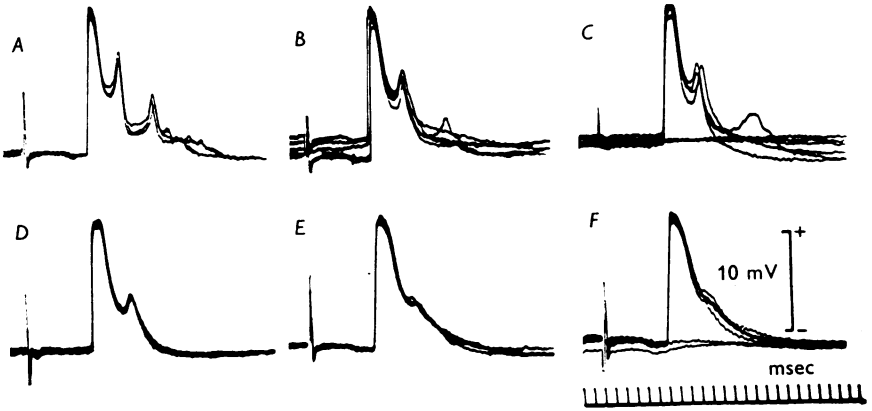

Fig. 5. Intracellular records from a Purkinje cell evoked by I.O. stimulation. The sequence illustrates the different responses which a single climbing fibre produced in a slowly deteriorating cell. In *A* the synaptic activation produced a series of small responses on the declining phase of the large depolarization. These responses are well synchronized at *A*, and fade out as the cell deteriorates, so that at *F* there remains virtually only the early potential with a more or less smooth decay. Note the demonstration of the all-or-nothing nature of this response in *C* and *F*.

In deteriorating Purkinje cells inferior olive stimulation may evoke in all-or-nothing manner complex depolarizing potentials, as in Fig. 5, that suggest repetitive synaptic action. However, the sequence of Fig. 5*A–F* shows that these complexities diminished and virtually disappeared as the cell further deteriorated; hence it can be concluded that they arose not from delayed synaptic bombardments, but from local responses that disappeared as the spike generating mechanism was progressively depressed by the falling membrane potential.

However, there are genuine cases of delayed synaptic bombardment of Purkinje cells in response to a single stimulation of the inferior olive. In Fig. 6*A* a just-threshold stimulation evoked either an EPSP with a triple summit having a latency of 7·3 msec and successive peaks at about 2 msec intervals, or a double-peak EPSP having a latency of 9·0 msec. With further increase in the stimulus strength (*B*) the latency was identical with the shorter value in *A*, and with still further strengthening there was further reduction in latency, to 6·0 msec in *C* and to 4·4 msec in *D*. At the same

time it will be noted that there was virtually no change in the latency of the potential (lower traces) simultaneously recorded from the surface of the folium within 1 mm of the recording micro-electrode. The initial negative peak of the surface potential had a latency of about 5·5 msec throughout, and its latency of onset was as brief as 2·6 msec in *D*. It seems that the weaker stimuli to the inferior olive were exciting presynaptic pathways to the cell of origin of the climbing fibre, and that only with the strongest stimuli (*D*) was this cell directly excited.

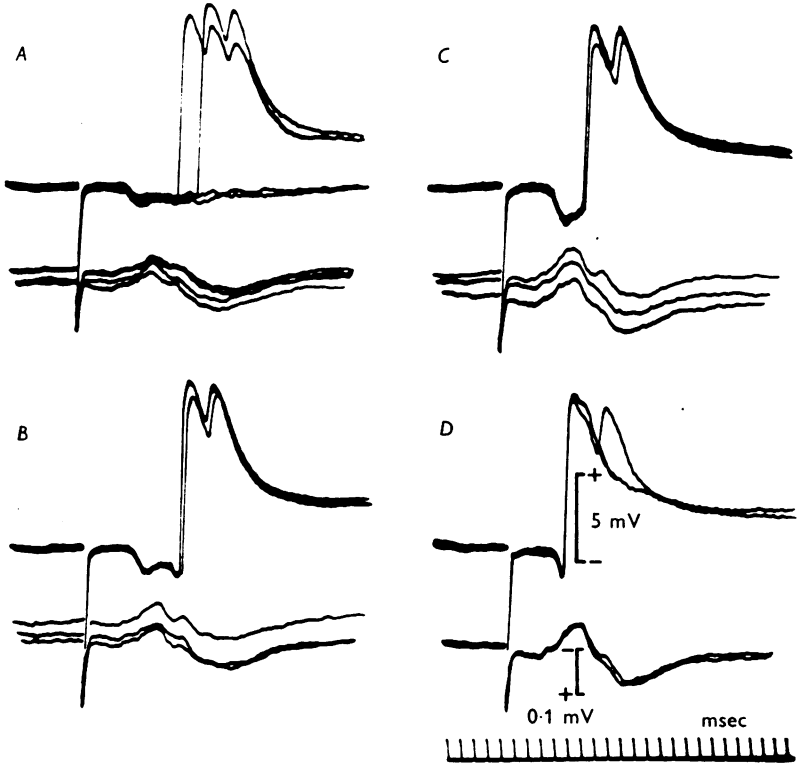

Fig. 6. Intracellular record from a Purkinje cell showing different latencies for the synaptic potential evoked by I.O. stimulation. Lower records are the simultaneous potentials recorded at the surface of the cerebellar cortex with their own potential scale. The stimulus strength was increased from *A* to *D*. Note in *A* and *D* the all-or-nothing nature either of this synaptic potential or of its later component. Timing of stimulus shown by downward shock artifact.

One explanation of these step-like variations in latency could be the existence of several sequential relays in the presynaptic pathway, there being presumably the jump of one synapse in the earlier record of Fig. 6*A*. Alternatively, the axons of inferior olivary cells may have collaterals that excite other olivary cells. Actually there is evidence in Figs. 10 and 11 for

such positive feedback. The discharge of some olivary cells could in this way excite others and these in turn still others; hence the latency of discharge along a particular climbing fibre may include several recurrent relays, and these can be by-passed in serial manner as in Fig. 6 as the stimulation is progressively increased.

It must further be postulated that the repetitive peaks are attributable to the repetitive discharge of the olivary cell that provides the climbing fibre to the Purkinje cell under observation in Fig. 6. Such a repetitive discharge at about 500/sec in response to a single presynaptic volley is very frequent with the inferior olive (cf. Figs. 8, 12), and has been observed with many other types of synaptic transmission.

The projection from the inferior olive to Purkinje cells. The pathway from the inferior olive to the cortex of the cerebellum traverses the contralateral restiform body (Brodal, 1954) and those climbing fibres going to the vermis should pass close to the fastigial nucleus. This expectation is confirmed by our finding that juxta-fastigial (J.F.) stimulation frequently evokes responses in Purkinje cells which are identical with those that have already been identified as being produced by stimulation of climbing fibres (CF) by means of the electrode in the inferior olive. Similarly Granit & Phillips (1956) found that juxta-fastigial stimulation often evoked large and prolonged depolarizations of Purkinje cells, and these 'D potentials' are now identifiable as climbing fibre responses.

In the extracellular records of Fig. 7*A–C*, graded J.F. stimulation evoked when weak the antidromic spike response of a Purkinje cell (*A*). In *B* the stimulus was just straddling threshold for a complex response, consisting of an initial spike potential in the cell that was antidromically invaded and a later series of two or three small spikes (*C*), the whole complex resembling that evoked a little later in that same cell by inferior olive stimulation (Fig. 3*D*).

Figure 7*D* gives another example of juxta-fastigial stimulation evoking first an antidromic spike potential and then a later spike superimposed on a prolonged potential. Subsequently the micro-electrode impaled this same Purkinje cell, and the juxta-fastigial stimulation then evoked (Fig. 7*E*, *F*) an initial antidromic spike potential and about 3·6 msec later a similar spike potential followed by a large depolarization on which small spikes were superimposed. These responses closely resemble those illustrated in Fig. 4*A–D* from two different experiments. In fact Fig. 4*B* and *C* provide two other examples of the responses evoked by climbing fibre stimulation by a J.F. electrode, the J.F. stimulus in *C* being below threshold for the axon of the Purkinje cell. The illustrations of intracellular CF responses are seen to conform to a standard pattern, which has been regularly observed when the Purkinje cell was in good condition.

Just as with the responses evoked by inferior olive stimulation, deterioration of the Purkinje cell leads to a diminution and eventual suppression of the spike components of the depolarizing response, so that eventually only a unitary post-synaptic potential remains. For example, in Fig. 8*A* the J.F. stimulation set up an initial antidromic spike with a later complex depolarizing response much as in Fig. 7*E*, *F*, but later the spike response of the cell had almost failed, so that there was no longer antidromic invasion, and the complex depolarizing response (*B*) consisted of an EPSP on which were superimposed two partial spike responses as in Fig. 5.

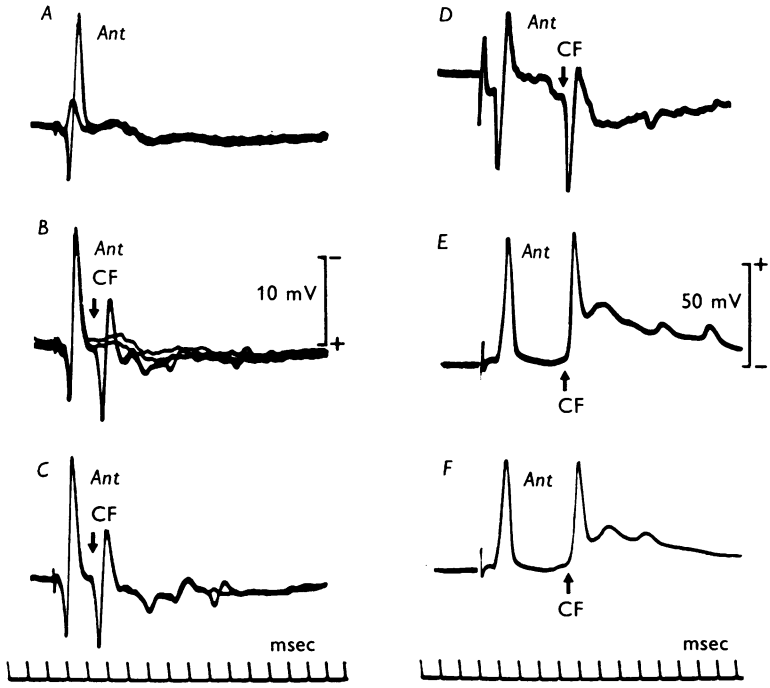

Fig. 7. Purkinje cell response to J.F. stimulation. In *A*, *B* and *C* extracellular spikes were evoked by J.F. stimulation at different strengths. In *A* the stimulus strength was straddling threshold for the antidromic activation (*Ant*) of the cell. In *B* a small increase to the stimulus strength produced a second response in an all-or-nothing manner (CF). This response, lasting about 7 msec, is remarkably similar to that produced in the same cell by I.O. stimulation (Fig. 3*D*). In *C* a further increase in the stimulus strength evoked both responses on every occasion. In *D* are the extracellularly recorded responses from another Purkinje cell that also were evoked by J.F. stimulation and that closely resemble *C*. In *E* and *F* are intracellular records of the same cell showing, after the antidromic invasion, a second spike (arrow) followed by a long-lasting depolarization (about 9 msec). The late potentials evoked by the J.F. stimulation in *B* to *F* are produced as indicated (CF) by the activation of a single climbing fibre. The potential scales of *B* and *E* are for the extracellular and intracellular records respectively. Time scale of *C* also applies to *A* and *B*, and that of *F* to *D* and *E*.

Further deterioration eliminated these partial spikes and there remained merely a unitary EPSP (Fig. 8*C*). In Fig. 8*D–F*, J.F. stimulation is seen to evoke a unitary EPSP superimposed on an EPSP–IPSP sequence that had a lower threshold. It will be shown in a later paper that this background EPSP response of the Purkinje cell is graded in size and is attributable to the excitation of mossy fibres by the J.F. stimulation, with the sequential excitation of granule cells and so to the parallel fibre synapses on the Purkinje cell dendrites.

The latency of the unitary responses. The unitary depolarizations attributable to climbing fibres were produced by juxta-fastigial stimulation with latencies ranging from 1·2 to 5·0 msec, there being only two examples out of almost 100 in which longer latencies (5·5 and 6·6 msec) were observed. This range of variation from 1·2 to 5·0 msec would be accounted for at least in part by variations in the location of the J.F. stimulating electrode,

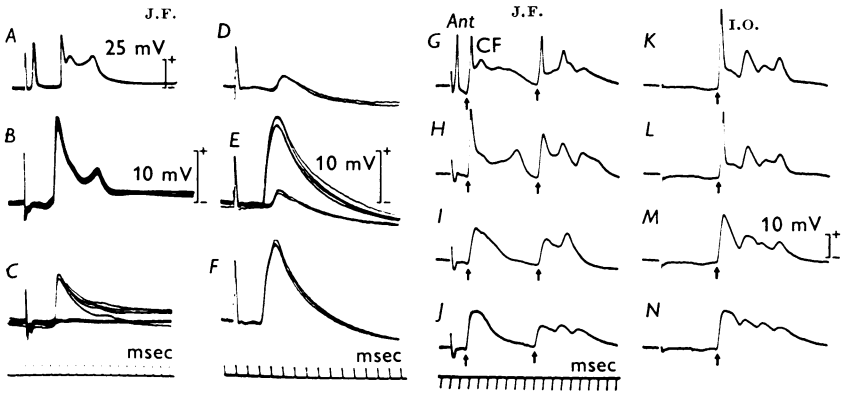

Fig. 8. Intracellular records evoked in deteriorating Purkinje cells by J.F. stimulation. *A*, *B* and *C* illustrate three stages of Purkinje cell deterioration. In *A* the cell fired antidromically, and the climbing fibre produced the usual spike followed by the large depolarizing potential. *B* shows an intermediate stage in which there was failure of the action potential mechanism so that there remained only the response to the climbing fibre—an excitatory post-synaptic potential with partial spikes on the summit and falling phase. In *C*, with further depolarization, the climbing fibre produced only an all-or-nothing synaptic potential, the amplification being the same as for *B*. *D*, *E* and *F* illustrate the intracellular potential evoked in a cell by different J.F. stimulation strengths. When the stimulus strength was raised, the all-or-nothing climbing synaptic potential appeared (note the latency difference). In *F* a stronger stimulus invariably produced a climbing fibre synaptic potential, the notch in the rising phase showing the superposition of the parallel fibre synaptic potential (cf. *D*). The production of the small EPSP in *D* by the parallel fibres is explained in the text. Records *G–N* show a Purkinje cell in the process of deterioration; potentials *G–J* and *K–N* were evoked respectively by J.F. and by I.O. stimulation, the records being arranged in the serial order of the state of deterioration. In records *G–J* the second arrow marks the 'reflex' activation of the climbing fibre by J.F. stimulation. Same potential and time scales for *G–N*. Time scale for *A*, *B* and *C* shown in *C*, and for *D*, *E* and *F* in *F*.

which would considerably affect the length of the climbing fibre to the Purkinje cell under observation. However, in most experiments the latency range for CF activation of adjacent Purkinje cells varied by a factor of more than two for a *fixed position* of the J.F. electrode, so presumably the range in conduction velocity of the excited climbing fibres is largely responsible for the latency range. As described in the next section, juxta-fastigial stimulation also often sets up delayed unitary responses that closely resemble the initial one and such a delayed response may have a lower threshold than the initial. Probably such delayed responses account for the two exceptionally long latencies reported above.

When comparison was possible between the unitary responses evoked by inferior olive and juxta-fastigial stimulation, they were found to be virtually identical, as may be seen in Figs. 4*A–D* and 7*E*, *F*. The only differences of significance were in the respective latencies, which almost invariably were briefer for the juxta-fastigial stimulation, and in the repetitive character often observed for the response evoked from the inferior olive (Figs. 6, 8*N*). In Fig. 4*B* and *C* the latencies were 4·9 and 5·0 msec, as against 6·1 msec for *A*.

Figure 8*G–N* gives a further illustration of the latency differential between climbing fibre responses evoked from the J.F. (*G–J*) and inferior olive (*K–N*) stimulating electrodes. In *G* the initial antidromic spike was followed by the CF response having a latency of 1·75 msec, while in the equivalent inferior olive response (*K*) the latency was 6·2 msec. In *G* there was also a second CF response at the arrow with a latency of 9·3 msec, which will be considered in the next section. As the condition of the Purkinje cell deteriorated in *H* to *J* and in *L* to *N*, the spikes failed, there being in *H* failure of the antidromic spike before the spike of the CF response. The latencies of the responses evoked by the J.F. and inferior olive stimulation remained at 1·75 msec (*H–J*) and 6·2 msec (*L–N*), respectively. In *J* and *N* the responses were simplified to EPSPs, which however were repetitive at about 500/sec (cf. Fig. 6) in the response from the inferior olive (*N*) and in the delayed J.F. response in *J*.

The differential latency for the two modes of stimulation (inferior olive and J.F.) has been measured for 79 Purkinje cells in which the complex depolarizing response could be evoked by both methods of stimulation. In 59 cells the J.F. response was between 1·0 and 3·6 msec briefer, while in twelve cells the differential latency was between 4·5 and 7·6 msec; and in eight cells it was less than 1·0 msec, approximating to zero in two. Since the actual conduction distance between the two sites of stimulation was about 20 mm (range approximately 17–23 mm in different experiments), conduction velocities of 4·7–23 m/sec can be calculated for the nerve fibres concerned in the latency differential of 1·0–3·6 msec.

Wide ranges of stimulation strength through either the inferior olive or J.F. electrodes almost invariably failed to disclose the convergence of two climbing fibres on to a single Purkinje cell. But two out of more than 100 cells exhibited a clear superposition of two unitary responses as in Fig. 9*A–D*. The first and second are displayed in the threshold-straddling series of Fig. 9*A* and *C*.

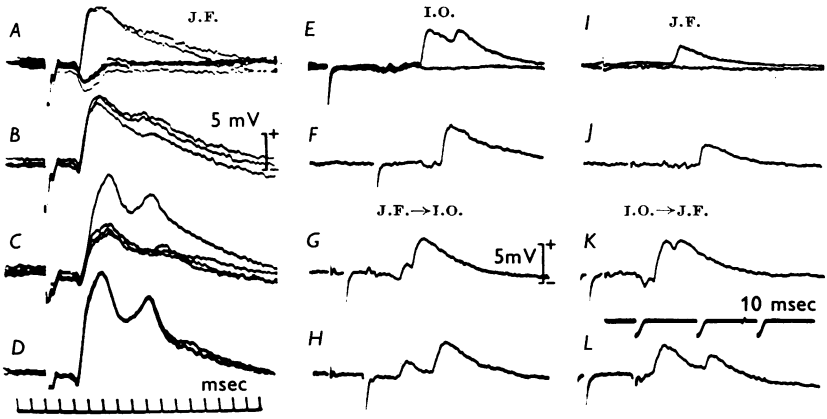

Fig. 9. Intracellular Purkinje cell records showing double CF innervation. *A* to *D* show four stimulation strengths for J.F. as described in the text. Records *E* to *L* illustrate responses of a Purkinje cell to two different climbing fibres, one being excited from the inferior olive (I.O. in *E*, *F*) and the other by juxta-fastigial stimulation (J.F. in *I* and *J*) as described in the text. Time scale in *D* and potential scale in *B* apply to records *A–D*. Time scale in *K* and potential scale in *G* apply to records *E–L*.

In the other example two quite different all-or-nothing EPSPs were evoked in a Purkinje cell by the inferior olive (Fig. 9*E*, *F*) and J.F. stimulations (*I*, *J*). With combined inferior olive and J.F. stimulations these two responses were summated at even the briefest stimulus intervals (*G*, *H*, *K*, *L*). Evidently the two modes of stimulation were exciting different climbing fibres that innervated the same Purkinje cell. In a subsequent paper (Eccles, Llinás, Sasaki & Voorhoeve, 1966) the interaction of the EPSP responses evoked by the same climbing fibre will be contrasted with the findings of Fig. 9*G*, *H*, *K*, *L*.

Other modes of activation of climbing fibres

Figure 10 shows at fast (*B–D*) and slow (*E–I*) speeds the double unitary responses (arrows indicating both first and second responses) often evoked in a Purkinje cell by a J.F. stimulation, there being an interval of 6·0–6·5 msec between the two responses both with extracellular (*B*) and intracellular (*C*, *D*, *E*, *F*, *G*) recording. Presumably the depressed size of the

second unitary response is attributable to its superposition on the residual depolarization following the first response. The double character of the synaptically evoked response in Fig. 10 is seen particularly clearly when deterioration prevented the generation of spike potentials by the neurone (*H*, *I*).

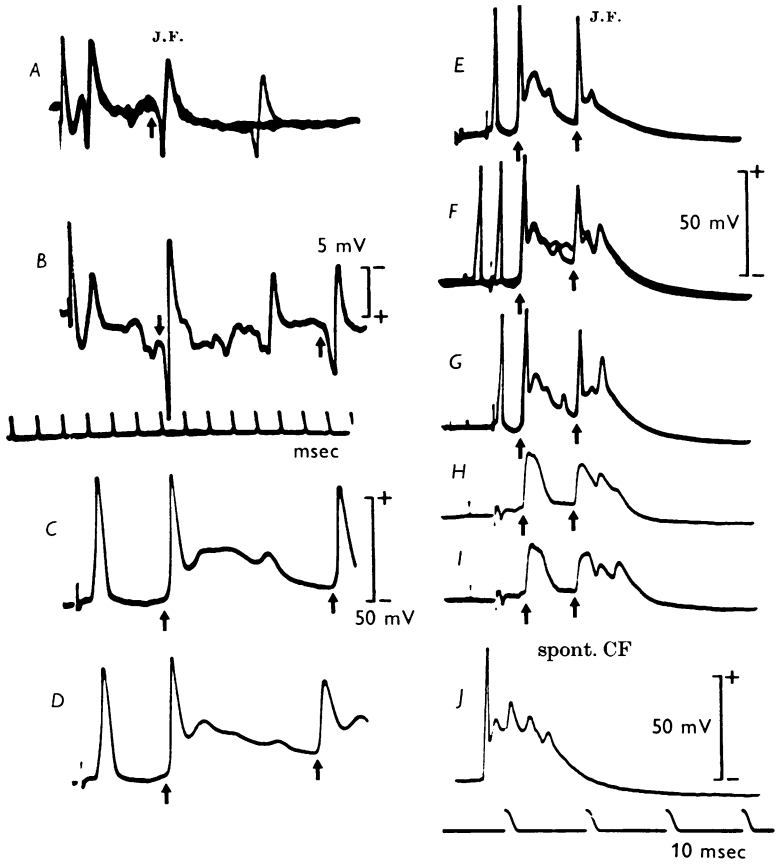

Fig. 10. Extracellular and intracellular Purkinje cell potentials evoked by reflex activation of a climbing fibre by J.F. stimulation. All records from *A* to *I* are from the same cell. In *A* and *B* extracellular Purkinje spikes show an initial antidromic activation, then the direct CF responses at the first arrows, and in *B* the reflex CF response at the second arrow. After impalement of the cell (*C*, *D*) there is the same sequence of the antidromic, then the direct and reflex CF responses at the arrows. *E*, *F* and *G* give the same responses at slower sweep speed. In *F* a spontaneous spike prevented the antidromic invasion of the cell in one of the two superimposed sweeps. Records *H* and *I* were taken after the spike generation had deteriorated and show the direct and reflex CF responses at the same latencies as in *E*–*G*. Some Purkinje cells gave spontaneous climbing fibre responses as in *J*. The potential scale of *B* applies also to *A*, that for *C* also to *D* and that of *F* to *E*–*I*. The same time scale for *A*–*D*. *E*–*J* are at the time scale below *J*.

Further examples of such delayed responses to J.F. stimulation are illustrated in Fig. 11. In *A*, J.F. stimulation evoked a climbing fibre response consisting of an initial spike trailing on to a smooth declining depolarization of several milliseconds duration. In *B–D* there was in addition a complex depolarizing response 8·2 msec later, which exhibited either a single EPSP (*B*) or a sequence of EPSPs (*C*, *D*) and which in *D* generated a second spike. These sequences of EPSPs closely resemble those evoked by inferior olivary stimulation (Figs. 6, 8*N*). The unitary character of this complex response is exhibited in the superimposed responses of Fig. 11*D*. In Fig. 11*E–H* from another experiment the delayed response usually had this sequence of EPSPs, which are shown in

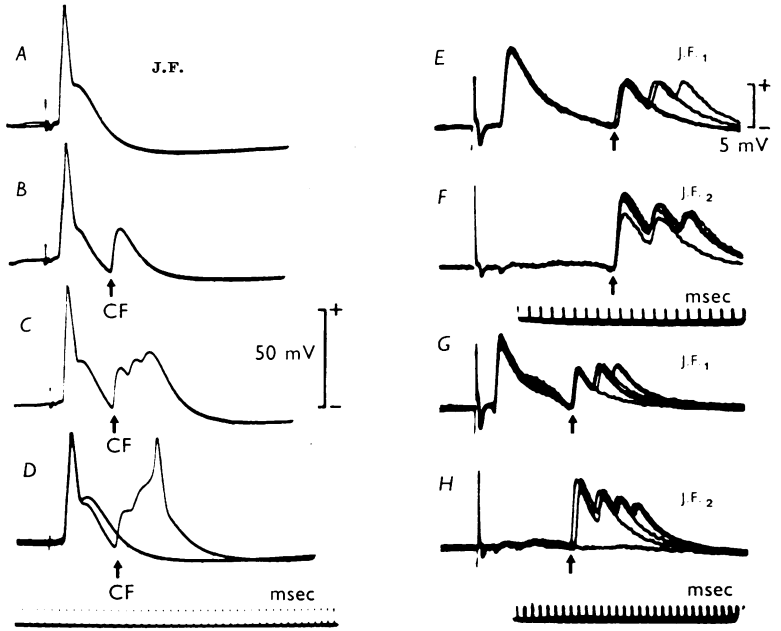

Fig. 11. Intracellular Purkinje cell potentials produced by direct and reflex climbing fibre activation by J.F. stimulation. In *A–D* the intracellular potential produced by direct CF activation was superimposed on the large IPSP produced by the simultaneous activation of inhibitory interneurones via the pathway—mossy fibres to granule cells to parallel fibres thence to basket and stellate cells. In records *B*, *C* and *D* the reflex activation of the CF at the arrows produced single and multiple synaptic potentials which summated and were able to generate a scond spike in *D*. Note that the amplitude of the synaptic potentials increased as they were evoked nearer the maximum value of the IPSP. Record *E* shows a direct and reflex EPSP by the climbing fibre. In *F* stimulation by another J.F. electrode (see Fig. 1) which did not stimulate this CF directly could nevertheless evoke the reflex activation of the CF at arrow. In *G* and *H* are similar potentials at slower speed. Potential scale of *C* and time scale of *D* apply to records *A–D*, potential scale of *E* applies to records *E–H*.

the superimposed records to appear at regularly spaced intervals, just as in Figs. 6 and 8*J*, *N*. In Fig. 11*E* and *G* stimulation through one J.F. electrode evoked an initial simple EPSP, as in Fig. 8*C*, and in addition the later response of 1, 2 or 3 EPSPs, as in Fig. 8*G–J*. In Fig. 11*F* and *H* stimulation by the other J.F. electrode evoked only the later complex response. Apparently this electrode was relatively further from the climbing fibre supplying the impaled Purkinje cell, which consequently was not directly excited. The pathways responsible for these delayed actions of climbing fibres will be considered in the Discussion.

In Fig. 12*A* and *B* a typical unitary climbing-fibre response of a Purkinje cell was evoked with a latency of 3·5 msec by stimulation of the inferior olive. The same unitary response with superimposed delayed components was evoked in *C* and *D* (note slower sweep speeds) by stimulation of the superficial radial and deep radial nerves of the ipsilateral fore-limb. The responses in *C* and *D* showed some latency variation, 12·2 and 13·2 msec in *C* and 11·5 and 12·9 msec in *D*. Figure 12*F* gives another example of climbing fibre stimulation from a peripheral nerve, the superficial radial. The unitary character of the response is seen in the several superimposed traces, which are extracellularly recorded from a Purkinje cell, the minimal latency being 17 msec. The same unitary responses were evoked from the inferior olive in Fig. 12*E* with a latency of about 5 msec. Most of the latency differential between the peripheral nerve and the inferior olive responses would be attributable to conduction time in the rather long neural pathway, though presumably there are one or more synaptic relays.

When strong stimulation was applied by an electrode on the surface of that folium into which the micro-electrode had been inserted, unitary responses of the typical climbing fibre character were sometimes observed to occur at a very short latency (Fig. 12*G*). Evidently, the strong stimulus had directly excited the climbing fibre supplying the Purkinje cell under examination.

It has been a common observation that Purkinje cells exhibit spontaneous responses that are identical with those evoked by CF stimulation (Fig. 10*J*). Presumably these responses are due to spontaneous discharges from inferior olive cells. Such spontaneous CF responses were also reported by Granit & Phillips (1956).

Effect of polarization of the Purkinje cell on the excitatory post-synaptic potential generated by climbing fibre impulses

If in its excitatory action the climbing fibre synapse acts in the usual manner by creating temporarily a high ionic permeability (Eccles, 1964, pp. 51–53), it should be possible not only to change the size of the EPSP

by altering the membrane potential of the Purkinje cell, but even to reverse the EPSP, as has been done with relatively few types of excitatory synapses (Coombs, Eccles & Fatt, 1955; Burke & Ginsborg, 1956; Nishi & Koketsu, 1960). In Fig. 13 the EPSP that is produced in a Purkinje cell

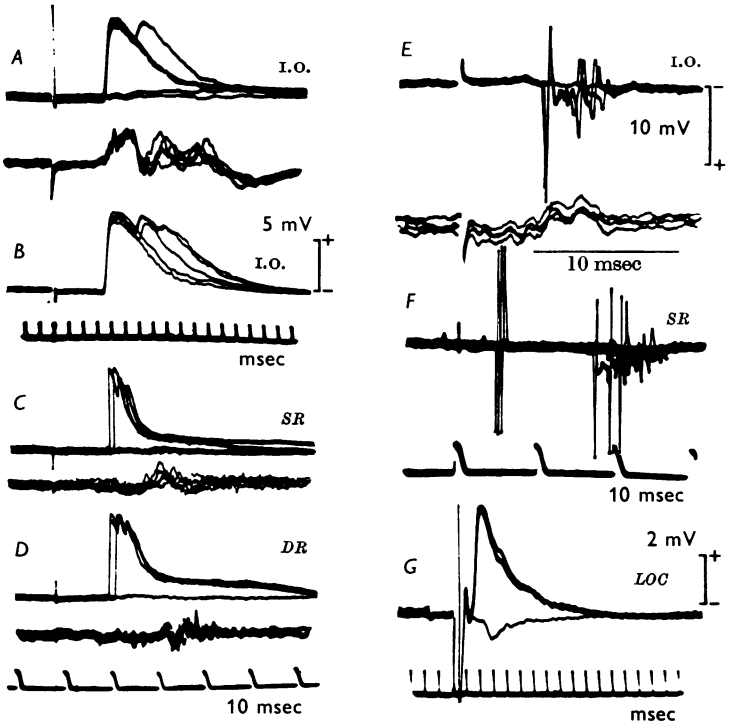

Fig. 12. Extracellular and intracellular Purkinje cell responses evoked by climbing fibres activated in various ways. *A* and *B* are intracellular potentials from a Purkinje cell showing the all-or-nothing CF synaptic potential evoked by I.O. stimulation, and the surface potential recorded simultaneously from the cerebellar cortex. In *C* and *D* all-or-nothing CF synaptic potentials were evoked in the same cell by stimulation of the ipsilateral superficial (SR) and deep radial (DR) nerves, respectively, the lower traces of each pair being recorded from the cerebellar cortex. Note the very prolonged synaptic depolarization indicating a prolonged transmitter action. The same amplification for *A–D*, but *C*, *D* at slower sweep. Record *E* shows the typical extracellular all-or-nothing Purkinje spike complex evoked by CF activation by the I.O. stimulus, together with the simultaneous record from the cerebellar surface. In *F* the same CF spike complex was evoked in the same cell by stimulation of the superficial radial nerve at 17 msec latency. In addition there was a single spike firing once in every sweep, at about 4·6 msec latency, which was evoked by the activation of the mossy fibre-granule cell-parallel fibre pathway. The same potential but different time scales for *E* and *F*. In *G* a CF all-or-nothing synaptic response was evoked in a Purkinje cell by local stimulation of the cerebellar cortex, the stimulating electrode (*LOC*) in Fig. 1 being in close proximity to the Purkinje cell under study.

by inferior olive stimulation (series *C*) is shown in *A* to have the typical unitary character of a climbing fibre synaptic action, and even to have sometimes the delayed additional responses (*B*). In Fig. 13*C* hyperpolarization of the Purkinje cell by a current applied through the recording electrode is seen to increase the EPSP, while a depolarization reduces and inverts the EPSP so that the climbing fibre synapse evokes a large hyperpolarizing potential. Figure 13 *D* and *E* illustrates in another cell even more

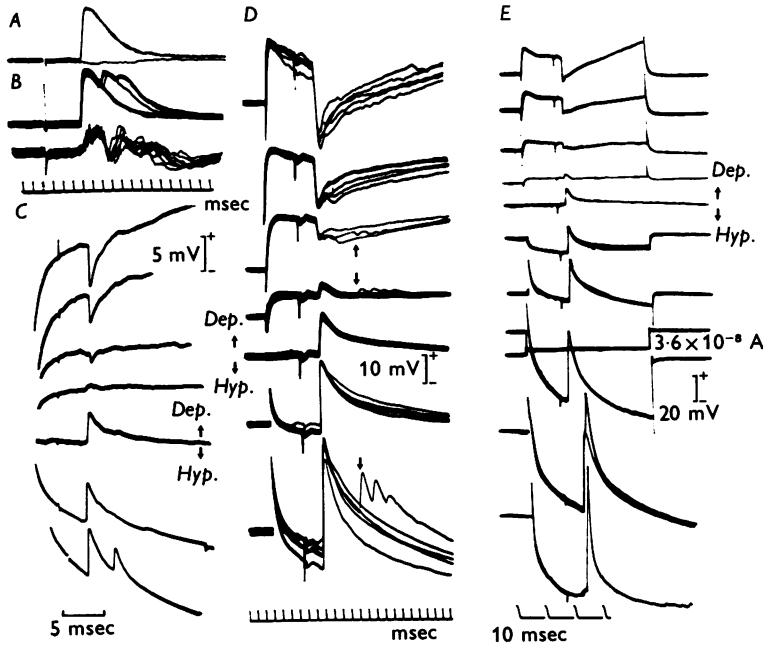

Fig. 13. Intracellular Purkinje's cell records of a CF synaptic potential modified by internally applied currents. Records *A* and *B* show the CF-synaptic potential (EPSP) evoked by I.O. stimulation together with the surface recorded response in *B*. In *C* an increase in the amplitude of the EPSP occurred when the hyperpolarizing current steps were applied through the impaling micro-electrode, while with depolarizing currents there was decrease and then reversal of the EPSP. The control synaptic potential is between the arrows. The same time and potential scales for *A–C*. In *D* are superimposed records of the CF-EPSPs evoked in another Purkinje cell by J.F. stimulation. As in record *C* depolarizing and hyperpolarizing currents had the same effects on the CF-EPSP. Note that the reflexly activated repetitive CF response of this cell (marked by the three arrows) was altered by the applied current in the same way as the directly evoked EPSP. Record *E* shows a second series of current applications to the same cell as in *D*, recorded at slower sweep speed and at lower gain. Reconstitution of the spike generating mechanism is observed when large hyperpolarizing currents were applied (lower two records). A current pulse $3{\cdot}6 \times 10^{-8}$ A corresponds to the third potential record downwards from the control response, which as in *C* and *D* is between the *Dep.* and *Hyp.* arrows.

remarkable examples of these large EPSP changes produced by changes in membrane potential. They were observed in two successive series of current application to the same Purkinje cell. Figure 13 *E* was recorded at lower amplification so that very large currents could be applied. It is remarkable that under such conditions the two largest hyperpolarizing currents could restore the impulse-generating property of the deteriorated cell and so increase the EPSP that it was able to generate a spike discharge, which is seen as an all-or-nothing event in the second lowest trace.

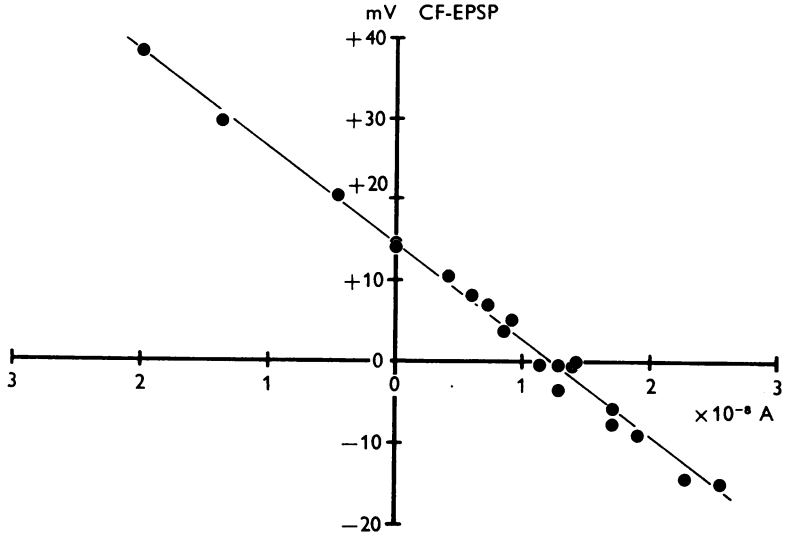

Fig. 14. Plot of the CF synaptic potential amplitudes (ordinates) against the intracellularly applied currents (abscissae). Note the straight line relation between the two.

It has not been possible to determine the membrane potentials produced by these applied currents; and, even if that had been possible, these measurements would obtain for the soma of the Purkinje cell and not for the region of the activated excitatory synapses on the dendrites. However, the applied currents were recorded, as may be seen in one trace of Fig. 13*E* for $3{\cdot}6 \times 10^{-8}$ A. The plotted points in Fig. 14 from the series partly illustrated in Fig. 13*E* are seen to lie along a straight line, there being an approximately linear relation between the applied currents and the EPSPs on either side of the reversal point that occurred with a depolarizing current of $1{\cdot}23 \times 10^{-8}$ A. Such linear relation has been observed in three of the six Purkinje cells in which this effect of current application on the EPSP has been investigated.

DISCUSSION

There is a remarkable correlation between the present electrophysiological observations on the powerful all-or-nothing synaptic activation of Purkinje cells and the classical histological findings of the very extensive synaptic contact that each Purkinje cell receives from a single climbing fibre. There is no evidence in conflict with the conclusion that impulses in climbing fibres are alone responsible for these uniquely large all-or-nothing responses of Purkinje cells that were originally observed by Granit & Phillips (1956). The physiological and histological evidence is further in agreement in showing that with rare exceptions (Fig. 9) only one climbing fibre is distributed to each Purkinje cell. Again, the conclusion of Szentágothai & Rajkovits (1959) that the very extensive olivo-cerebellar tract is exclusively composed of climbing fibres is fully corroborated by our evidence that apparently pure climbing fibre responses of Purkinje cells can be regularly evoked by stimuli applied to the inferior olivary nucleus.

Several illustrations have been given (Figs. 6; 8*N*; 12*A*, *B*; 13*B*) of the frequent observation that stimulation of the inferior olive evokes a brief sequence of all-or-nothing responses at about 2 msec intervals. These responses are identical with responses evoked by repetitive activation of a climbing fibre (Eccles *et al.* 1965); hence, presumably, they signal the repetitive discharge of impulses along a climbing fibre from its cell of origin in the inferior olive. The direct or short latency responses evoked by stimulation of the climbing fibre by the juxta-fastigial electrode (Figs. 8*C*, *E*, *F*, *I*, *J*; 10*H*, *I*; 11) have never exhibited this repetitive character; yet, as shown in Figs. 8*I*, *J*; 10*H*, *I*; 11*C–H*, it was frequently observed with the reflex responses following juxta-fastigial stimulation. With such reflex responses there must be synaptic excitation of the inferior olivary cells; so it can be concluded that, when similar responses are evoked by stimulation of the inferior olive, the stimulus is exciting presynaptic fibres in that nucleus. At many monosynaptic relay stations in the central nervous system it is now recognized that the synaptic excitatory action by a single afferent volley similarly evokes a brief repetitive discharge, as indeed occurs with climbing fibre activation of Purkinje cells (Figs. 3, 4).

When juxta-fastigial stimulation leads to the reflex production of CF responses, as in Figs. 8*G–J*, 10 and 11, the latent period of these responses is always sufficiently long for a reflex pathway through the inferior olive. There are two simple pathways by which juxta-fastigial stimulation could lead to the reflex discharge of impulses from the inferior olive. In one the axon collaterals of climbing fibres would excite synaptically cells of the inferior olive. Figure 11*F*, *H* shows that this positive feedback pathway through an axon collateral would have to go at least in part to cells other

than the cell of origin of that axon collateral. The alternative pathway would be antidromic transmission down mossy fibres and thence by axon collaterals to inferior olive cells. It has been recognized histologically that many fibres give axon collaterals to the inferior olive as they pass by (Ramón y Cajal, 1909; Scheibel & Scheibel, 1955; Scheibel, Scheibel, Walberg & Brodal, 1956) and many of these fibres could well be cerebellar mossy fibres.

However, this simple axon collateral pathway is not in itself capable of explaining the step-like shortening of latency that occurs in Fig. 6 with progressive increase in stimulus strength to the inferior olive, where actually four steps are illustrated. Only two can be accounted for by the postulate that the weakest stimuli excite fibres that are presynaptic to the cells of origin of the climbing fibres, while the stronger directly excite the discharge of impulses along the climbing fibres. In order to explain the additional latency steps, it is necessary to postulate either serially arranged interneurones in the inferior olive or a pathway via positive feedback collaterals which can go through several relays of inferior olive cells before reaching the cell of origin of the climbing fibre under observation. Direct investigation of the inferior olive is required in order to distinguish between these alternatives. Further investigation is also required before an explanation can be given of the very brief latency differential (less than 1·0 msec) that has sometimes been observed for the CF responses evoked by I.O. and J.F. stimulation respectively.

A remarkable feature of the Purkinje cell responses to climbing fibre activation has been the relatively long duration (about 5 msec) of the repetitive spike discharge evoked by a single impulse (Fig. 3*A*, *B*; 4*A–D*; 7*B*, *C*, *E*, *F*). By contrast, in the deeply deteriorated Purkinje cell (Figs. 5*F*; 8*C*, *E*, *I*, *J*; 10*H*, *I*; 11*E*, *G*; 13*A*) the EPSP shows a relatively rapid and smooth decay to a low slowly decaying residuum. Under such extreme conditions it can be assumed that the membrane time constant is greatly shortened and that these brief EPSPs with a prolonged tail give an approximate indication of the time course of the synaptic transmitter action. With Purkinje cells being recorded from intracellularly under good conditions, the depolarization produced by the synaptic excitation would have a longer duration; hence it seems possible to account satisfactorily for the rather long duration of the repetitive discharge of impulses observed under such conditions (Figs. 4, 5, 7, 10) or with extracellular recording (Figs. 3, 12*E*, *F*).

In Figs. 3*C*, 7*B–D*, 10*A* the spike potentials evoked by CF stimulation have a configuration (positive–negative diphasicity) that as usual closely resembles the antidromic spike potential. However, this resemblance merely shows that in both these cases the spike potential propagated into

the region of the Purkinje cell under observation which was the soma together with the large basal dendrites. It could be that the spike evoked by the CF impulse was generated in the axon and propagated into the soma-dendritic zone exactly as with the antidromic impulse set up by J.F. stimulation. A more probable alternative is that the CF impulse generated the spike in the dendrites in the region of its maximum synaptic action at about 150–200 μ below the surface. The propagation of this spike down the dendrites to the soma region would give the diphasic positive–negative spike observed in this region (Figs. 3*A–C*; 7*B–D*; 10*A*, *B*; 12*E*). However it must be recognized that the extracellular recording of giant spike potentials provides a misleading indication of the normal site of initiation of impulses. Local mechanical injury by the juxtaposed micro-electrode tends to suppress spike initiation; hence the invariable finding that such giant potentials begin with an initial positivity (Freygang, 1958; Freygang & Frank, 1959; Terzuolo & Araki, 1961).

The field potential profiles (Fig. 2) indicate that a CF impulse generates the first impulse discharge in the Purkinje cell dendrites at the region of greatest density of climbing fibre synapses. Thereafter the situation is less clear. Intracellular recording invariably shows that subsequent spike potentials are greatly depressed (Figs. 4*A–D*; 7*E–F*; 10*C*, *D*), which is of course attributable to the severe membrane depolarization by the intense synaptic excitation. With extracellular recording this depression is also observed, but the configuration of the extracellular spikes in Figs. 3*A*, *B*; 10*A*, *B*; 12*E*, *F* certainly shows that many grow into fully propagated impulses. Presumably when the spike production of the soma and dendrites is depressed by the heavy depolarization, spike discharge can still be generated in the axon, just as is believed to occur for many types of nerve cell. This generation of repetitive axonal impulses would be expected to give the small spikes observed by intracellular recording. Possibly also some of these spikes are generated in the more remote dendrites.

The relatively slow time course of propagation of impulses in the slender climbing fibre branches is indicated by the increasing delay of onset of the negative field potential towards the surface (Fig. 2). However, this increase in conduction time would account for a range of at the most 2 msec in the onsets of the EPSPs produced by the many synapses made by a single climbing fibre. Hence the relatively long duration of transmitter action with the prolonged low level tail may be attributed to the slow diffusion of the transmitter away from the sites of release and of action, just as occurs, for example, in Renshaw cells (Eccles, 1964, p. 45).

As mentioned above, large and prolonged unitary depolarizations were observed by Granit & Phillips (1956) to occur either spontaneously or after juxta-fastigial stimulation. These responses were called by them

'inactivation responses' because of the suppression of spike generation during the strong depolarization. We would attribute all these responses to the very powerful synaptic excitatory actions of impulses in the climbing fibres. Furthermore, it is now clear from such records as those of Fig. 8*A–C* and Fig. 10*E–I* that with intracellular recording the typical inactivation response of Granit and Phillips is transformed into their typical *D* potential as the impaled Purkinje cell deteriorates so that spike generation is suppressed. A similar interpretation can be offered for the *D*-potentials reported by Suda & Amano (1964).

In their records of Purkinje cell potentials induced by juxta-fastigial and other types of stimulation Deura & Snider (1964) have recorded many complex potentials that may now be identified as typical climbing fibre responses. Juxta-fastigial stimulation often, as in Figs. 7, 8*A*, *G* and 10, evoked a complex spike potential that now can be recognized as a unitary CF response. Furthermore, with juxta-fastigial stimulation, they reported delayed responses that correspond with the reflex responses in Figs. 8*G–J*, 10*B–I* and 11, having a latency of about 6 msec longer than the direct responses. The changes in potential wave form with deterioration of the impaled cell (Figs. 5, 8, 10) were also illustrated by them. It is of particular interest that they found the same CF response (as it may now be called) to be evoked by cerebral as well as by J.F. stimulation. Evidently there is a fast pathway from the cerebral cortex (latency about 6 msec) for activation of climbing fibres.

According to the classical description of Ramón y Cajal (1911) a climbing fibre is distributed to only one Purkinje cell. On the contrary Scheibel & Scheibel (1955) report that a single climbing fibre may innervate adjacent Purkinje cells. Conceivably extracellular recording could provide additional evidence on this point. Innervation of adjacent Purkinje cells by a single climbing fibre would be established if the all-or-nothing climbing-fibre response produced at exactly the same threshold was composed of spike potentials from two Purkinje cells. This has never been observed. However, this negative finding does not exclude multiple innervation because only occasionally was the extracellular electrode located so that it could record from two Purkinje cells. In any case it must be emphasized that the present electrophysiological findings provide an excellent correlation with the usual histological picture, and reveal that the climbing fibre is the most powerful and specific excitatory synapse yet discovered in the central nervous system. Evidently each climbing fibre must have the function of providing specific probing in order to sample from instant to instant the integrated excitatory-inhibitory state of a Purkinje cell, and the whole climbing fibre mechanism can in this way be envisaged as a detailed pattern sampling device.

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